THE GENOTYPE HYPOTHESIS AND HYBRIDIZATION[1]

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It sometimes seems as if the hypercritical attitude had become an obsession among biologists. A proper judicial spirit is of course essential to science, but do not biologists often require a large amount of affirmative data before assenting to a proposition which is in reality a simple corollary of one already accepted?

For example, Darwin emphasized small quantitative variations as the method of evolution, although he recognized the occurrence of larger changes both quantitative and qualitative. De Vries, on the other hand, emphasized large variations—especially qualitative variations—as the real basis of evolution, although he too admitted the existence of lesser changes. He distinctly states that a mutation or new basis for fluctuating variation, may be so small that it is obscured by the fluctuations themselves.

If relative frequency of occurrence is a criterion of the value of variations in organic evolution, which is not necessarily so, Darwin's point of view is probably the nearer correct. If one could find a unit basis for describing variations in terms of the physiological economy of the organism concerned, *i. e.*, if one knew exactly what was a large change and what was a small change, he would probably find that a random sample of inherited variations followed the normal curve of error. By this I

[1] Read at the symposium on the "Genotype Hypothesis" at the meeting of the American Society of Naturalists, Ithaca, N. Y., December 28, 1910.

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mean that small variations would center closely around a mode, and large variations would occur with a relative frequency inversely proportional to their size. The point that I wish to emphasize, however, is that neither Darwin nor De Vries recognized the proper distinction between a mutation and a fluctuation. Darwin made no distinction. De Vries, however, considered fluctuations to be linear; that is, to be limited to increase and decrease in characters already present. He thought that selection of such variations brought about changes in the selected population due to the inheritance of the fluctuations, but that the selected populations returned to the mean of the general population after selection ceased. Mutations, on the other hand, were gains or losses of entire characters—qualitative changes—which were transmitted completely, *i. e.,* were constant, from the beginning. De Vries did indeed state that mutations could take place in any direction, which would involve the idea of linear change or quantitative mutations; yet it seems quite evident from his general attitude in ''Die Mutationstheorie'' that to his mind qualitative and quantitative variations were quite distinct.

Many practical breeders had long known, however, that the selection of linear variations often produced new races which were as constant as any races, provided they were not exposed to crossing with individuals of the general population from which the selected race had come. Why this was true was unknown. It was felt that there was a real distinction between certain variations, to which Darwin had not called attention; yet it was felt that the De Vriesian idea was not wholly correct. It has been in making this distinction clear-cut and definite that Johannsen has rendered his great service. His elaborate extensions of the genotype conception of heredity have cleared up many debated points, and corroborative evidence has been received from so many lines that it can hardly be doubted that the main points of the hypothesis are correct. It may seem, therefore,

as if the superstructure of this conception were too elaborate to rest upon a simple foundation; yet I can not see but that the basis of the entire hypothesis is the fact that a fluctuation is a non-inherited variation produced upon the soma by environmental conditions, while the inherited variation, the mutation if you will, is any variation qualitative or quantitative, that is germinal in character. This being so, it seems scarcely necessary for an elaborate proof of the proposition, for it is nothing but a corollary to that part of Weismannism which was already generally accepted.

Of course it is recognized that pure Lamarckism still has followers to whom neither Weismannism in any form nor the genotype conception of heredity could appeal. But to thorough Weismannians and to those who believe in occasional germinal response to environmental conditions, it seems as if both propositions must be acceptable and their interdependence apparent.

Let us follow this line of reasoning to its logical conclusion in regard to the physiology of heredity. The Mendelian notation has been generally accepted as a convenient way of accounting for the facts of heredity in certain markedly discontinuous characters. It has been questioned by many, however, whether the Mendelian conception is not rather an apparent interpretation of a relatively small number of facts than a general law. De Vries has even suggested that there are definite physiological reasons why certain characters should Mendelize and others should not. His idea is that Mendelian segregation occurs when a germinal determinant for a character (*Anlage*) meets an opposing determinant, and when no such opposition exists the character in the cross-bred organism breeds true. Now the universal tendency of the facts of breeding is towards an interpretation the opposite of this. When a determinant from one parent meets with no such determinant from the other parent (presence and absence hypothesis), Mendelian segregation appears. When the same determinant is received from

both parents, segregation can not be proved, for the character breeds true.

In fact the many results of experimental breeding during the past few years have convinced me that De Vries's general conception of this matter is incorrect. There may be finally a considerable modification of our ideas regarding the ultimate nature of Mendelian unit characters and the exact meaning of segregation, yet the universal applicability of a strict Mendelian system to interpret the facts of heredity becomes more and more apparent every day. And the point that I wish to emphasize is that Mendelian inheritance is a genuine corollary of the genotype hypothesis if the latter is applicable to a population in a state of natural hybridity. In my work with maize where free intercrossing does occur I am convinced of the existence of genotypes in a state of natural hybridization. Furthermore, these genotypes can be isolated by inbreeding. If it were true, then, that only certain markedly discontinuous characters such as color Mendelize, how could genotypes which differ from each other in size characters be isolated? It is not expected, however, that the statement that Mendelian inheritance and the genotype hypothesis are interdependent will be received without proof. Data that are believed to furnish such proof are submitted here.

When Mendelism was a new idea it was natural that the behavior of many hybrids should be regarded as irreconcilable to such a system of interpretation. The earlier criticisms arose largely through the misconception that dominance instead of segregation was its essential feature. Later, when so many complex results from pedigree cultures were fitted into a strict and simple Mendelian notation, it was objected that the investigators could by expert juggling of a sufficient number of factors interpret according to their system any experimental results they might obtain. Perhaps a few biologists regarded as a personal affront the gradual growth of the idea that the facts of heredity were complex, but it is

hardly likely that many could regard this complexity as
an invention of Mendelians. The latter would only too
gladly have the facts as simple as possible.

There have remained, however, several instances in
which hybrids apparently did not segregate in the F_2
generation. Mendel himself investigated one such case,
the genus *Hieracium*. The investigation of Ostenfeld[2]
made this case perfectly clear by showing that the hy-
brids reproduced apogamously. Such asexual reproduc-
tion may also explain the behavior of hybrids between
species of brambles which are also said to breed true in
all their characters. These cases, however, and others
among animals of which human skin color is the example
par excellence, may be left out of consideration because
no exact data concerning them have been forthcoming.
There remain the experiments of two careful investi-
gators who observed no segregation in the F_2 generations
of their hybrids, those of Lock[3] upon heights of maize
plants and those of Castle[4] upon weights and ear lengths
of rabbits. Lock expected that if segregation occurred
it would be into two classes, *i. e.*, simple mono-hybridism.
For this reason he made no measurements which would
show whether he obtained the kind of segregation which
as is shown later in this paper, does occur in maize hy-
brids. Castle[5] has recently admitted the possibility that
his numbers were not large enough to prove definitely
that segregation involving several small unit characters
does not occur in the ear length of rabbits.

The difficulty attending this earlier work was that there
was no way of explaining different manifestations of the
same character. Segregating characters could always be
interpreted either as the presence and absence of a unit

[2] Ostenfeld, C. H., 1904, ''Zur Kenntnis der Apogamie in der Gattung
Hieracium,'' *Ber. Deutsch. Bot. Ges.,* 22: 7.

[3] Lock, R. H., 1906, '' Studies in Plant Breeding in the Tropics,'' III,
Experiments with Maize, *Ann. Roy. Bot. Gard. Peradeniya,* 2: 95–184.

[4] Castle, W. E., *et al.*, 1909, ''Studies of Inheritance in Rabbits,'' Car-
negie Inst. Wash. Pub., 114: 5–70.

[5] In lectures at the Lowell Institute, Boston, Mass., 1910.

giving a $3:1$ ratio, or as the complemental action of two different units each allelomorphic to its absence, giving $9:3:3:1$ ratios or modifications of them. Nillson-Ehle[6] and the writer,[7] however, have shown that several units each allelomorphic to its own absence may be the determinants of what appears to the eye as a single character. In the above paper the writer suggested that if such ratios as $15:1$ and $63:1$—di-hybrid and tri-hybrid ratios, respectively—were found in considerable numbers, then higher ratios of this kind might account for the apparent constancy of hybrids in characters that seemed to be continuous. For, if—as is quite probable—the additional units increase the activity of the character in question, and if there is no dominance,[8] it is quite evident that hybrids may be intermediate between the two parents. All the pure classes in a complex character of this kind would indeed be difficult to isolate, but segregation could be absolutely proved by a comparison of the variability of the F_1 and F_2 generations.

Since writing the above paper I have obtained clear evidence of $15:1$ ratios in two other cases. The first is a red pericarp color, the second is the condition of endosperm in maize which gives dented seeds as distinct from that which gives flinty seeds. There is even considerable probability that higher ratios occur which affect the latter character. In another paper[9] I have shown photographic evidence of size segregation in varieties of *Nicotiana rustica* and stated that similar evidence of segregation of size character in maize had been obtained. The following figures and tables show sufficient of the evidence from the maize crosses to demonstrate conclusively

[6] Nillson-Ehle, H., 1909, "Kreuzungsuntersuchungen an Hafer und Weizen," *Lunds Universitets Årsskrift*, N. F., Afd. 2., Bd. 5, Nr. 2. 1–122.

[7] East, E. M., 1910, "A Mendelian Interpretation of Variation that is Apparently Continuous," AMER. NAT., 44: 65–82.

[8] One dose, *i. e.*, receiving the same gene from a single parent, would on the average increase the manifestation of the character half as much as two doses.

[9] East, E. M., 1910, "The Rôle of Hybridization in Plant Breeding," *Pop. Sci. Mon.*, Oct., 1910, pp. 342–354.

that size characters segregate. It is hoped that this evidence will make us more cautious about accepting uncorroborated statements about characters which are definite exceptions to the Law of Mendel. It is by no means certain that no such exist, but no experimental proof of hybrids non-Mendelian in character has been made.

A further proof of segregation of size characters has recently been made in a preliminary note by Emerson.[10] He states that definite segregation occurs in beans, gourds, squashes and maize. His full data are therefore awaited with great interest.

Table I shows the frequency distribution of the heights of plants in a cross between no. 5 a medium-sized flint maize and no. 6 a tall dent maize. Sufficient seed was obtained in a previous season so that the entire series could be grown in rows side by side during one summer. This procedure eliminates any possibility that the variability of the F_2 generation might have come from varying conditions of soil fertility.

It will be noticed that the F_1 generation is nearly as tall as the taller parent. This increase in size is not due to dominance. It is the increased vigor that comes from crossing in maize, and while it obscures the hereditary differences in size, it is really a problem of development and not of heredity as was shown in a previous paper.[11]

The distribution of heights in the F_2 generation is seen by simple inspection of the table to be more variable than the F_1 generation in the case of each ear planted. Reduced to simple terms by the calculation of the coefficient of variation in each case, however, the two generations can be compared more accurately. In the F_1 generation the C.V. $= 8.68 \pm .553$ while in the various F_2 generations from different ears the coefficients of variation run from $12.02 \pm .559$ to $15.75 \pm .684$.

[10] Emerson, R. A., 1910, ''Inheritance of Sizes and Shapes in Plants,'' Amer. Nat., 44: 739–746.

[11] East, E. M., 1909, ''The Distinction between Development and Heredity in Inbreeding,'' Amer. Nat., 43: 173–181.

TABLE I [12]

FREQUENCY DISTRIBUTION OF HEIGHTS OF MAIZE PLANTS IN CROSS (5 × 6)

No.	51	54	57	60	63	66	69	72	75	78	81	84	87	90	93	96	99	102	105	108	111	A.	S.D.	C.V.
5	3	2	4	5	11	17	17	18	10	6	4											68.22 ± .406	6.49 ± .324	9.51 ± .421
6													1	2	2	13	14	16	14	8	3	101.18 ± .400	5.07 ± .283	5.01 ± .279
(5 x 6)− F₁												4	3	9	9	6	5	4	4	7		94.53 ± .740	8.21 ± .519	8.68 ± .553
(5 x 6)− 1 F₂	2	1	3	6	3	5	10	10	22	16	5	7	11				8	4				81.00 ± .685	12.76 ± .486	15.75 ± .684
(5 x 6)− 2 F₂				2		2	4	2	6	5	10	8		13	6	10	3	10	4		2	88.33 ± .802	11.78 ± .567	13.34 ± .675
(5 x 6)− 8 F₂					4	4	12	7	9	13	12	15	12	8	8	1	1	1	8	2		79.46 ± .621	9.55 ± .438	12.02 ± .559
(5 x 6)−14 F₂	2	1	2	4	2	7	8	17	21	11	27	24	16	9	11	10	5	4	2	2		81.24 ± .513	10.28 ± .364	12.65 ± .450

Class Centers in Inches for Heights of Plants

TABLE II [13]

FREQUENCY DISTRIBUTION OF HEIGHTS OF PLANTS IN CROSS (54 × 60)

No.	22	25	28	31	34	37	40	43	46	49	52	55	58	61	64	67	70	73	76	79	82	85	88	91	Total
No. 54							→[14]						2	6	10	17	24	26	21	14	14	7	3	1	145
No. 60	←————————→[14]																								100
(60-5 x 54) F₁	←————→[14]																								55
(60-8 x 54) F₁																					→[14]				26
(60-3 x 54) F₁																						→[14]			30
(60-5 x 54) F₂ [15]	←							1	8	18	26	44	95	117	126	92	59	31	22	3	3				649
(60-8 x 54) F₂ [16]								1	5	6	13	13	35	29	36	27	21	20	9	6	4	1	1		228
(60-3 x 54) F₂ [17]						5	15	13	32	31	68	60	72	34	47	14	13	5							409

Class Centers in Inches for Heights of Plants

[12] Grown side by side in same season.

[13] Distributions giving figures grown side by side in same season.

[14] Extremes were measured and number of plants counted. All were strongly convergent around the central classes.

[15] Grown from five different ears.

[16] Grown from two different ears.

[17] Grown from three different ears.

Table II shows a similar distribution of heights in cross between no. 60, a dwarf pop maize commonly known as Tom Thumb, and no. 54, a sugar corn known as Black Mexican. The distribution of heights of no. 54 was obtained in the same season as the F_2 generation. They were both grown upon the same plot of ground in which the soil appeared to be quite uniform. Unfortunately, the exact distribution of the heights of no. 60 and of the F_1 plants which were grown in previous seasons, is unknown. The range of the variates shown by the black lines, however, is correct. Furthermore, from notes recorded at the time we know that the F_1 generation was comparatively uniform, the greater number of variates being distributed around classes 67, 70 and 73 inches. In this case, also, the effect of crossing is shown by the relatively high plants of this generation. The plants of the F_2 generation show a wide range of variation. The highest individuals are practically the height of the highest individuals of the taller parent, no. 54. The lowest plants of F_2 do not reach the lower range of no. 60. I interpret this as due to continued heterozygosis in other characters and to physiological correlation. By the latter term I mean that since the plants of no. 60 are very small, F_2 segregates of the same size could only be expected where the ears and seeds also are very small. But since the ears and seeds of these plants also show segregation in new combinations, normal growth correlation probably resulted in a somewhat larger average size. For example, little 40-inch plants were found with ears three times the length of normal ears of no. 60. It is likely that such plants might have been smaller if they had been recombined with the characters necessary for the production of smaller ears.

Table III and Figs. 1–4, show the lengths of ears in the cross just described. In making this table the best ear from each plant that bore a well-filled ear was taken. The small ears, therefore, do not represent poor, unfilled or supernumerary ears. The coefficients of variability

TABLE III [18]

FREQUENCY DISTRIBUTION OF LENGTHS OF EARS IN CROSS (60 × 54)

Class Centers in Cm. for Lengths of Ears

No.	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	A.	S.D.	C.V.
No. 60	4	21	24	8														6.6 ± .073	.81 ± .051	12.27 ± .783
No. 54									3	11	12	15	26	15	10	7	2	16.8 ± .121	1.87 ± .088	11.13 ± .531
(60–5 × 54) F_1 [19]					1	12	12	14	17	9	4							12.1 ± .121	1.51 ± .088	12.48 ± .722
(60–5 × 54) F_2 [20]			4	5	22	56	80	145	129	91	63	27	17	6	1			12.7 ± .058	1.99 ± .037	15.67 ± .296
(60–8 × 54) F_2 [23]			1	10	19	26	47	73	68	68	39	25	15	9	1			12.9 ± .076	2.25 ± .053	17.44 ± .413
(60–3 × 54) F_2 [21]			2	5	17	33	33	33	27	21	13	10	11	12	1	2	1	12.6 ± .128	2.81 ± .087	22.30 ± .744

TABLE IV

FREQUENCY DISTRIBUTION OF WEIGHTS OF SEEDS OF CROSS (60 × 54)

Class Centers in Grams for Weights of 25 Seeds

No.	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6.0	6.5	7.0	7.5	8.0	8.5	9.0	9.5	10.0	10.5	11.0	11.5	A.	S.D.	C.V.
60	7	22	28	4																	2.7 ± .034	.39 ± .024	14.44 ± .899
54									2	2	5	7	14	10	5	4	1	2	1	2	8.3 ± .107	1.21 ± .074	14.54 ± .963
(60–5 × 54) F_1 [22]				5	12	13	17	4	3												4.6 ± .059	.64 ± .041	13.91 ± .912
(60–5 × 54) F_2 [22]				17	49	98	113	83	69	43	27	13	8	1		1					5.3 ± .032	1.09 ± .022	20.56 ± .442
(60–8 × 54) F_2 [23]					3	15	16	17	23	18	18	12	8	9	1	1	2				6.2 ± .067	1.23 ± .047	19.84 ± .799
(60–3 × 54) F_2 [24]				4	10	24	41	53	40	52	21	19	9	8	2	3					6.0 ± .047	1.17 ± .033	19.50 ± .563

[18] No. 60 and F_1 gen. grown in 1909, No. 54 and F_2 gen. in 1910.

[19] Grown from five ears.

[20] Grown from two ears.

[21] Grown from three ears.

[22] Grown from five ears.
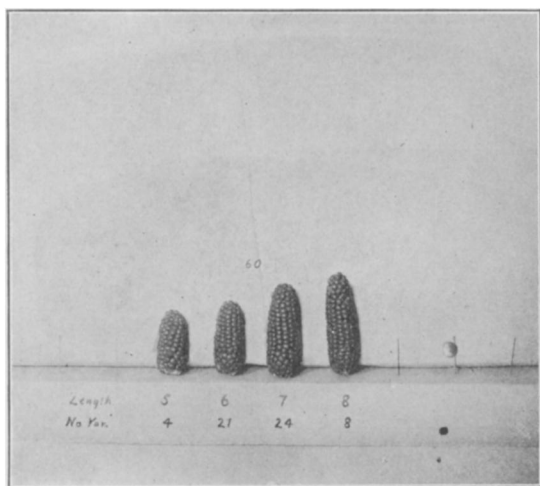
[23] Grown from two ears.

[24] Grown from three ears.

FIG. 1.   No. 60, female parent, illustrating variation in length of ear ($\frac{1}{8}$).

have again been calculated, but they hardly emphasize
the real segregation as well as do the photographs which
were made from representative ears of the different
classes found in the actual crop.

Table IV shows the segregation of weights of seeds in
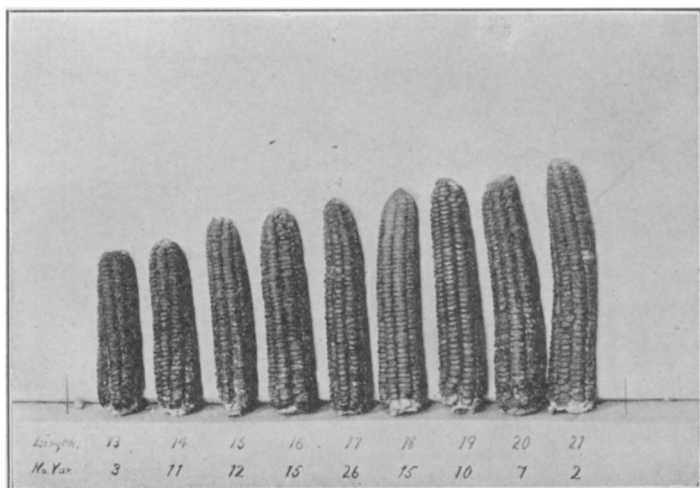F_2 in this same cross.   Fig. 5 shows the average size of

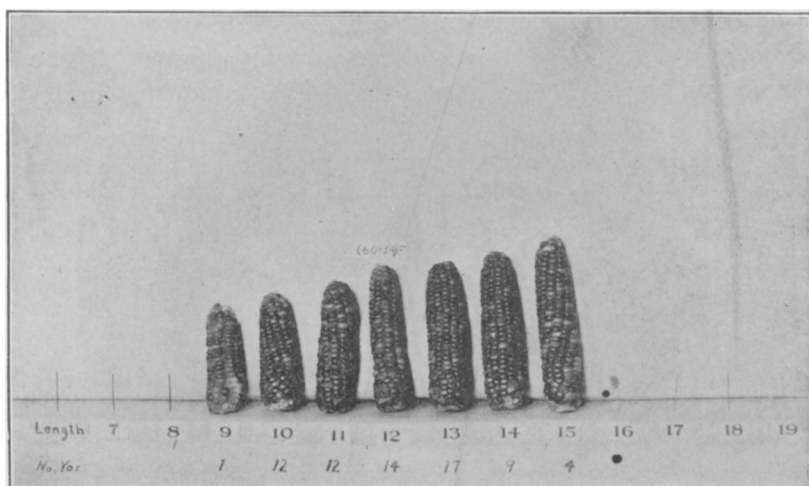FIG. 2.   No. 54, male parent, illustrating variation in length of ear ($\frac{1}{8}$).

FIG. 3.   Variation in length of ear of F_1 generation of cross between
No. 60 and No. 54 ($\frac{1}{8}$).

the seeds of the two parents and the F_1 generation and
the extremes of the F_2 generation.   In making the
weights for this table, it was necessary to use a scheme
by which the sugary wrinkled seeds of the Black Mexican
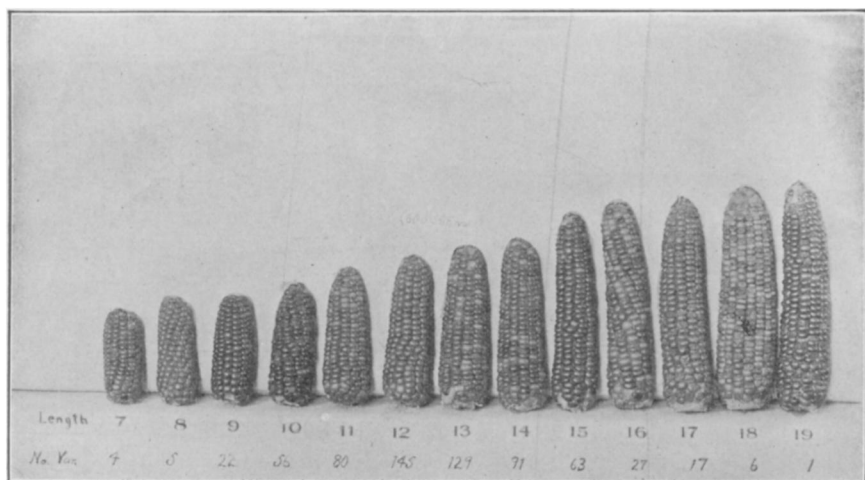parent, no. 54 could be weighed as starchy seeds.   This

FIG. 4.   Variation in length of ear of F_2 generation of cross between
No. 60 and No. 54 ($\frac{1}{8}$).

was done by planting the no. 54 between rows of the hybrid. Sufficient crossed seeds which had become starchy through *Xenia* were obtained to make the weights given. Not all of the ears, however, had 25 starchy seeds, which accounts for the small number of plants measured. Furthermore, the seeds of no. 54 were a rather mixed lot, which of course resulted in a higher variability than would probably have been found if only seeds
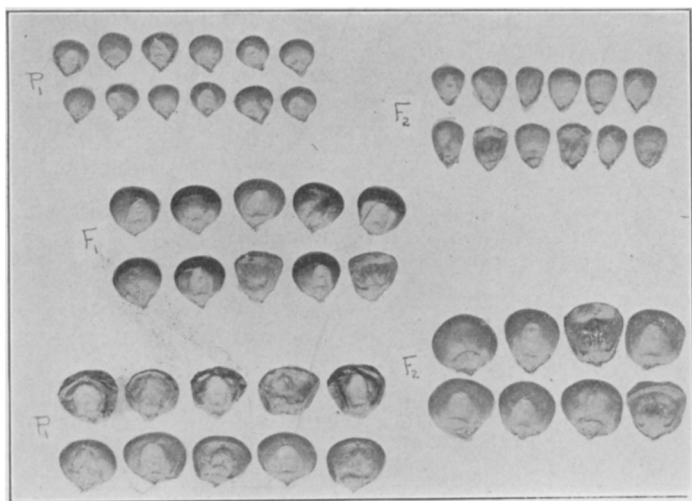

FIG. 5.   Average size of seeds of No. 60 and No. 54 and the F_1 generation of the cross between them.   Extremes of the F_2 generation.

of the individual plant of no. 54 which was used as the male parent of the cross, could have been planted. Perhaps it should be noted here since the question might arise, that since the size of the seeds on an ear is governed by the development of the pericarp, the sugar corn, no. 54, was unaffected in other ways than by having the pericarp filled out with starch by the hybridization which occurred attended by the resultant *Xenia*.

In Tables III and IV the measurements and weights of the F_1 generation were recorded from only one cross, although three crosses between the two varieties were made. It might be said that one has the right to com-

pare only the F_2 generation of cross of which the F_1 generation is given. If this were granted our conclusions in regard to segregation would be the same. It might be said, however, that sufficient records were made of the F_1 generations of the other crosses to know that they differed but little from the family of which the data were recorded. In addition, it is a fact that general
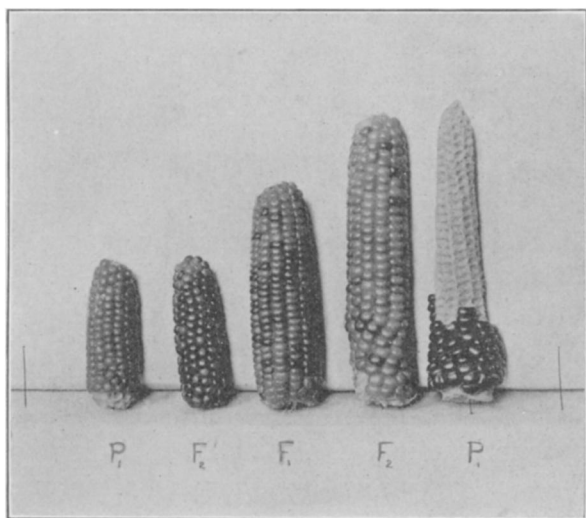

FIG. 6. Average ears of No. 60 and No. 58 and the F_1 generation of the cross between them. Extremes of the F_2 generation.

populations of the two parents were studied, and their variation was undoubtedly greater than would have been that of the inbred progeny of the three parent plants of either variety.

An additional cross between Tom Thumb pop maize and a small purple flint is illustrated in Fig. 6. The ears pictures show the average size of the two parents and the F_1 generation, and extremes of the F_2 generation.

In conclusion there are two points I wish to notice. Unquestionable segregation in size characters has been shown by comparison of the F_1 and F_2 generations. It can scarcely be doubted that some of these segregates will breed as true as the parent forms, yet one can

scarcely do more than speculate in regard to the specific characters that are concerned in developing either organs or individuals of certain sizes. There are probably many characters that interact together in developing certain characters, although the actual determinants in the germ cells may be transmitted independently. These interdependent reactions during development obscure to us the real causes and what we regard as independent characters may be but indirect results of unknown causes. For example, the ability to evert their starch when heated has been the distinguishing character of the subspecies called *Zea mays everta,* the pop maizes. This character so called, however, is the resulting physical condition of the starch caused at least partially by the small size, the thickness and the toughness of the enveloping pericarp.

For these reasons it may not be possible—at least very soon—to point out even the number of characters concerned in size developments. From the number of extreme segregates obtained in each case I might venture to state that the size of ear in the cross shown in Fig. 5 is apparently due to not less than three characters, while the size of ear in the other cross pictured seems to be due to not less than four characters.